

PARTIAL PURIFICATION OF THE B-ESTERASE OF BOVINE MILK

Tenfold purification of the B-esterase (1) of bovine milk has been obtained by adsorption of the enzyme on a $Mg(OH)_2$ suspension, followed by decomposition of the $Mg(OH)_2$ to a soluble carbonate with carbon dioxide under pressure and removal of ions by dialysis.

Details of the purification procedure were as follows: Milk known to be high in B-esterase activity was selected. It was withdrawn from the glass jar at the milking parlor into a brown glass bottle, immediately placed in ice water, brought to the laboratory, and gassed with nitrogen (2). It was stored in an atmosphere of nitrogen, at 2-5° C., until the following day. All subsequent operations, except assays for enzyme activity and nitrogen, were conducted in the cold room (2-5° C.). One hundred and sixty milliliters of gravity skim, obtained from the above milk, were added to 40 ml. of $Mg(OH)_2$ suspension (3) in a 250-ml. centrifuge bottle and shaken vigorously for 30 sec. The mixture was then centrifuged (800 g) for 10 min., the supernatant discarded, and the sides of the centrifuge bottle rinsed with water while the bottle was inverted. Forty milliliters of water was added to the bottle, the $Mg(OH)_2$ pellet was dispersed by shaking and again centrifuged down (15 min. at 800 g). Again, the supernatant was discarded. The $Mg(OH)_2$ was immediately transferred to a stainless steel pressure cylinder by rinsing the centrifuge bottle with three successive 30-ml. portions of water. After flushing with carbon dioxide, a pressure of 30 p.s.i. of this gas was applied for 30 min., during which time the cylinder was continuously shaken in a horizontal plane. At this point, if the $Mg(OH)_2$ was completely decomposed, the preparation foamed considerably when the pressure was released. A trace of foam depressant was added, the liquid transferred to cellophane sacs, and dialyzed against repeated changes of water for 48 hr. During

the above steps, an effort was made to keep the time of contact between enzyme and $Mg(OH)_2$ to a minimum.

Degree of purification was calculated on the basis of esterase activity per milligram of nitrogen. Esterase activity was estimated using Warburg manometric techniques (1), at pH 8.0 and 37° C., with tributyrin as substrate. The reaction mixture contained eserine sulfate (10^{-5} M) to inhibit any cholinesterase present. One milliliter of heated milk ($75 \pm 5^\circ$ C. for 10 min.) was added to reaction mixtures when the purified preparation was being assayed. Nitrogen content of the skim milk and esterase preparations were determined by a semimicro-Kjeldahl method.

Five-to tenfold purification of the B-esterase of milk has been obtained repeatedly, using the methods described above. Two recent preparations showed an average purification of 10.7-fold and a yield of 44% of the original activity. Several preparations have been lyophilized with only slight loss in activity. One of these shows no activity loss after storage at 2-5° C. for 2 mo.

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SIMPLE METHOD FOR EXTRACTING FAT FROM WHOLE MILK POWDER

In connection with a study of off-flavor constituents in foam-dried whole milk powder, a simple, rapid method has been developed which quantitatively extracts the fat from both foam-dried and spray-dried powders. Previous work in this regard has been recorded by Lampitt and Bushill (2) and Whitney and Tracy (3). However, both of these methods were deemed unsuitable for our purpose, mainly because of the relatively long procedural times involved.

EXPERIMENTAL PROCEDURE

Fifty milliliters of methanol (Baker's¹ ACS

¹The mention of trade names in this paper is for identification and implies no endorsement of the products.

grade) and 20 g. of powder were mixed with a glass rod until fluid enough (about 5 min.) to be stirred magnetically (15 min.). The methanol extract was filtered from the powder through S & S 507 paper using light suction. The dry powder was returned to the beaker and stirred with 100 ml. n-hexane (Phillips,¹ high grade) for 15 min. The slurry was filtered as above and the hexane extraction repeated once more. All solvents were removed on the steam bath, the residues taken up in about 75 ml. of methylene chloride (Fisher,¹ highest purity), dried over anhydrous sodium sulfate, and filtered through sintered glass into tared vessels. After removal of the solvent, the fat was dried to constant weight in a vacuum desiccator. For routine analyses it is not, of course,

TABLE 1
Recovery and distribution of fat from whole milk powders *

Whole milk powder	Type	Trial No.	Total fat recovered *	Per cent of total fat recovered in		
				Methanol	1st Hexane extraction	2nd Hexane extraction
			(%)			
Commercial a	Spray	1	99.58	9.67	88.02	2.31
		2	99.70	5.14	92.57	2.29
USDA	Foam (2 mo. old)	1	95.94	6.62	86.78	6.59
		2	96.12	6.69	85.92	7.38
USDA	Foam (2 yr. old)	1	96.72	7.30	83.29	9.40
		2	96.78	8.32	85.56	6.11
Commercial b	Spray	1	96.81	6.54	91.53	1.92
		2	93.88	5.75	89.74	4.50
USDA	Foam (fresh)	1	97.52	13.37	85.59	1.04
		2	97.71	21.03	76.36	2.61

* Recoveries based on the average of closely agreeing duplicates obtained by the Mojonnier method.

necessary to weigh the individual extracts. It is done here solely for the purpose of showing the fat distribution in each extract. In practice, the extracts may be combined and evaporated.

RESULTS AND DISCUSSION

Results of the study are presented in Table 1. The wide variation in the amount of fat obtained in any one extraction probably is due to differences in the amount of vacuum applied, more fat being carried into the methanol filtrate mechanically with higher vacuum. The end result, however, is the same as manifested by the good agreement between duplicates.

Scaling the procedure up (100 g. powder) resulted in a yield of 99% on fresh foam-dried powder. No recovery studies were made on less than 20 g. powder. The procedure appears flexible enough to permit the use of solvents other than n-hexane, although we did not investigate this point. Substituting n-propanol for methanol gave very poor yields. The use of hexane purified by the method of Bassette

(1) furnished fat with no detectable solvent odor, which ostensibly can be used for organoleptic evaluation.

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DICHLOROFLUORESCEIN AND POTASSIUM CHROMATE AS INDICATORS IN A TITRATION TEST FOR SALT IN CHEESE ¹

The common laboratory procedure used for measuring salt in cheese is the modified Volhard method, described in 1937 by the Committee on Cheese Analysis of the American Dairy Science Association (A.D.S.A.) (6).

A direct titration method for measuring the salt content of butter was proposed by Sammis (3) for factory practice. Marquardt (2) adapted the Sammis technique to the analysis for salt in a water dispersion of cheese and compared this test for salt in cheese with the modified Volhard method. It was found that the

extent of proteolysis of cheese adversely affected the direct titration, because water-soluble and suspended protein in concentrations above 0.2 g. per 100 ml. of solution introduced an error proportional to the amount of protein in the aliquot tested. Consequently, it was recommended that the test be used only for cheese less than five days old. Potassium chromate and dichlorofluorescein (DCF) produced comparable results when used as end point indicators in this direct titration method.

Skelton and Bryant (4) and Weckel (5) found that dichlorofluorescein gave a sharper end point and greater accuracy than potassium chromate in the direct titration test for salt in butter.

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